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ApoE Genotype Does Not Affect Plasma tPA and PAI-1 Antigen Levels

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The presence of one or two apoliprotein E4 (apoE4) alleles constitutes a major risk factor for Alzheimer's disease (AD) and coronary heart disease (CHD). Numerous observations have suggested that misregulation of proteases may be instrumental in both diseases. Tissue-type plasminogen activator (tPA) has been recently demonstrated to play a key role in neuronal plasticity and in experimental neurodegeneration. One receptor for the ApoE protein is the LRP/α2 macroglobulin receptor, which also binds to and endocytoses tPA and plasminogen activator inhibitor I (PAI-1). Here we tested whether the apoE genotype has an influence on the plasma levels of these proteins. We demonstrate that there is no difference in plasma levels of tPA- and PAI-1-antigens between middled-aged individuals with one apoE4 allele and those having none. This suggests that the impact of apoE4 on Alzheimer's disease is not the result of altered clearance of tPA or PAI-1 by the LRP receptor. Am. J. Med. Genet. 74:172–175, 1997.

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INTRODUCTION

Numerous studies have demonstrated an influence of the apolipoprotein E (apoE) genotype on late-onset sporadic and familial Alzheimer's disease (AD) [Corder et al., 1995; Poirier, 1994], as well as on coronary heart disease (CHD) [Davignon et al., 1988]. ApoE is a 34-kD protein involved in plasma lipoprotein metabolism, cholesterol homeostasis, and local lipid transport. Polymorphism at two sites within the apoE gene in humans results in three main allelic variants (ϵ2, ϵ3, and ϵ4). Although homozygotes for the ϵ4 allele are rare in the general Caucasian population (2–3%), their number is increased eight times in AD patients and up to 16 times in subjects under age 40 years with CHD [Van Bockxmeer and Mamotte, 1992].

The strong impact of apoE4 allele in both CHD and AD could reflect some common basic molecular mechanisms underlying both diseases. In accordance with this hypothesis, pathological alterations in the cerebral microvasculature have been reported in neurodegenerative diseases such as AD, which affect cortical layers particularly prone to degeneration [Buee et al., 1994; de la Torre, 1994; Kalaria and Hedera, 1995]. Also, in Alzheimer's brains, there is a reduced level of serpin nexin-I, a major regulator of thrombin in the central nervous system (CNS), which suggests that an imbalance in thrombolytic regulators exists in this pathology [Wagner et al., 1989]. Finally Skoog et al. [1996] showed the association between elevated blood pressure at age 70 and the development of dementia 10–15 years later.

ApoE binds the LDL receptor-related protein (LRP, also known as the α2-macroglobulin (α2M) receptor). LRP is a multifunctional clearance receptor that binds the tissue plasminogen activator (tPA), either free or complexed, to the plasminogen activator inhibitor-I (PAI-1) as well as to complexes of α2M with proteases [Krieger and Herz, 1994]. tPA cleaves plasminogen into plasmin and is inhibited by PAI-1. Since ApoE and tPA share a common receptor, the apoE genotype might influence indirectly the activity of fibrinolytic enzymes. Changes in the fibrinolytic system, which are regulated by the balance between the relative concentrations of tPA and PAI-1, occur in pathologies associated with thrombotic episodes. Elevated plasma levels of PAI-1 and tPA have been demonstrated in CHD and are a well-accepted risk factor in myocardial infarction [de Bono, 1994; Salomaa et al., 1995] and stroke [Ridker et al., 1994]. High levels of tPA also appear to be deleterious for neurons, since it was shown that neurons from tPA null mice are protected from excitotox-

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icity induced by kainic acid [Tsirka et al., 1995], a treatment known to upregulate tPA gene expression in the brain.

We investigated the possibility that the apoE genotype influences the tPA/PAI-1 balance in serum and thereby contributes to the deterioration of the vascular system and to the neuronal death seen in AD. To test this, we examined the impact of apoE alleles on plasma concentrations of tPA, PAI-1, and α2M. We measured the concentrations of these proteins in the blood from 134 healthy donors and determined the apoE genotype of each individual. Within this healthy population we found, as expected, clear positive correlation between plasma tPA- and PAI-1 antigen (ag) levels. tPA- and PAI-1-ag levels also correlated positively with body mass index and age, while α2M correlated negatively with both criteria and with tPA ag. We showed that there is no impact of the ε4 allele on the levels of any of these proteins, suggesting that the deleterious effect of the ε4 allele is not due to an abnormal level of circulating protease-related LRP ligands in ε4-bearing individuals.

MATERIALS AND METHODS

Sampling

Sera were obtained from 134 healthy individuals at rest using the procedures described by Kluft and Verheijen [1990]. In brief, blood samples were taken from fasting, resting (20 min), lying individuals. In order to avoid the effects of circadian rhythms, samples were all collected at 9:30 AM ± 30 min, and only from individuals with a normal day/night rhythm preceding the sampling. Blood was collected in glass tubes containing citrate as anticoagulant. The venipuncture was made after a minimal stasis, using a vacuum technique. The first 5 ml of blood were discarded. The blood was kept on ice until centrifugation (30 min at 2,000*g* at 4°C). The plasma aliquots were frozen at −20°C until measurement. Samples were coded to ensure anonymity.

DNA Purification

DNA was purified from leukocytes using the QIA Ampblood kit (Qiagen #29104).

ApoE Genotyping

ApoE genotyping was determined by *Hha*I restriction polymorphism on DNA produced by polymerase chain reaction (PCR) amplification of the ApoE gene using the forward primerTCCAAGGAGCTGCAGGCGGCGCA and the reverse primer TCGGCGGATGGCGCTGAGGCCG. The product of restriction analysis was analyzed by electrophoresis on 20% acrylamide gels and by visualization of the DNA fragments with ethidium bromide staining. Genotypes were assigned by comparing the patterns of fragments to those obtained with DNA of plasmids coding for the three ApoE genotypes [Wenham et al., 1991].

tPA and PA-I Antigens

The tPA and PA-I antigens were measured using enzyme immunoassays provided in the commercial TintElize tPA-1 kit (Biopool #101120) and TintElize PAI-1 kit (Biopool #210221). Standard deviations on the measurements were 9%, and tests were carried out in duplicate.

Alpha 2 Macroglobulin Activity

The α2 macroglobulin concentration was measured by a commercial kit based on the anti-trypsin activity of α2 macroglobulin. The concentration was calculated based on a 3.7-μM standard provided by the supplier of the kit (Coaset, Chromogenix, Mölndal, Sweden). Standard deviations of the measurements were 12%, and tests were carried out in duplicate.

Statistics

The Mann-Whitney test was used to analyze differences between groups. The Spearman rank correlation was used for all correlations shown in Table II. *r* is the Spearman *r*. *P* values given in the text and in the tables are two-tailed *P* values.

RESULTS

The data of this study were analyzed in several ways. Demographic characteristics of the 134 healthy donors are given in Table I, and the mean values for circulating levels of tPA antigen, PAI-1 antigen, and α2 macroglobulin are grouped according to ApoE genotype. The frequencies of the three common alleles of the apoE gene were similar to those reported by others in normal Caucasian populations: ε2, 0.056; ε3, 0.825; ε4, 0.119.

Table II shows the correlations between variables obtained using the Spearman correlation. A strong

TABLE I. Descriptive Summary of Sample Population Stratified by ApoE Genotypes*

Genotype	Total population	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
Number	134	12	3	91	27	1
tPA (ng/ml)	7.2 ± 3.3	9.0 ± 3.8	11.1 ± 0.8	7.0 ± 3.3	6.3 ± 2.7	8
PAI-1 (ng/ml)	10.6 ± 10.5	14.5 ± 11.3	24.1 ± 17.8	10.5 ± 11.0	7.8 ± 5.4	11.6
α2M μM	4.5 ± 1	4.4 ± 1.1	4.1 ± 0.8	4.5 ± 1.0	4.5 ± 1.0	5.6
Age (years)	34.6 ± 7.4	36.4 ± 8.7	41.7 ± 6.7	34.3 ± 7.5	34.2 ± 6.1	
BMI	22.2 ± 2.5	23.8 ± 1.7	23.2 ± 3.9	21.9 ± 2.5	22.2 ± 2.6	24.7
Weight (kg)	66.5 ± 11.6	74.9 ± 8.5	70.3 ± 12.5	65 ± 11.4	65.4 ± 12.2	
Height (cm)	172.4 ± 9	177 ± 7.6	174 ± 15.6	172 ± 9.1	171 ± 7.9	
Male, female	68, 66	10, 2	1, 2	46, 45	10, 17	1

*Values are mean ± SD, tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor; α2M, alpha 2 macroglobulin; BMI, body mass index.

TABLE II. Spearman Correlations Between Protein Levels, Age and BMI*

	tPA	PAI-1	α2M
tPA			$r = -0.35$ $P < 0.0001$
PAI-1	$r = 0.8175$ $P < 0.0001$		$r = -0.199$ $P = 0.02$
Age	$r = 0.4127$ $P < 0.0001$	$r = 0.2444$ $P = 0.0047$	$r = -0.3159$ $P = 0.0002$
BMI	$r = 0.4814$ $P < 0.0001$	$r = 0.4412$ $P < 0.0001$	$r = -0.3224$ $P = 0.0001$

*tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor; BMI, body mass index.

positive correlation was found between tPA levels and PAI-1 levels.

Table III shows the very significant differences seen between genders for tPA (8.6 ± 3.2 ng/ml for males, 5.6 ± 2.6 ng/ml for females, $P < 0.0001$).

Comparing individuals carrying at least one ϵ4 allele (ϵ4+) and those carrying none revealed no significant differences in levels of tPA, PAI-1, and α2M.

In contrast, a significant difference ($P = 0.0069$) was found between the means of blood tPA concentrations from individuals carrying one or two ϵ2 alleles and those carrying none (9.4 ± 3.5 ng/ml, and 6.8 ± 3.2 ng/ml, respectively). Grouping the ApoE2-carrying individuals by sex (Table III) showed that there was no significant difference in tPA levels within males between ϵ2+ (n = 11) and ϵ2– (n = 57) (9.2 ± 3.6 ng/ml, and 8.5 ± 3.1 ng/ml, respectively). However, ϵ2+ females (n = 4) had significantly higher tPA levels than ϵ2– females (n = 62) (10.3 ± 3.6 ng/ml, and 5.3 ± 2.2 ng/ml, respectively; $P = 0.0181$). PAI-1 levels were also significantly higher in ϵ2+ females than in ϵ2– females (23.8 ± 16.2 ng/ml, and 6.1 ± 4.3 ng/ml, respectively; $P = 0.0249$). Residual tPA activity, as estimated by the difference between PAI-1-ag and tPA-ag-concentrations, was also significantly higher in ϵ2 females (13.6 ± 13.5 ng/ml, and 0.8 ± 3.0 ng/ml, respectively; $P = 0.023$).

α2 macroglobulin concentrations were not significantly different between either ϵ4+ and ϵ4– groups, or between ϵ2+ and ϵ2– groups.

DISCUSSION

In this study we examined the possible relationship between the ApoE genotype and the circulating levels of tPA-antigen, PAI-1-antigen, and α2 macroglobulin, in the blood taken from resting, healthy, middle-aged donors. The mean circulating tPA- and PAI-1-ag were significantly higher in men than in women. The very small standard error in tPA measurements within these groups reflected the reproducibility of the sampling.

As observed by others, we detected a very strong positive correlation between the levels of tPA and of PAI-1 [Mussoni et al., 1992]. There were also strong positive correlations between tPA, PAI-1 concentrations and Body Man Index (BMI), and age. Our study also demonstrated a weak but highly significant negative correlation between α2 macroglobulin levels and BMI, age, and tPA.

An unexpected finding of our study was the apparently strong impact of ϵ2 on tPA and PAI-1 levels in females. The mean tPA level of this ϵ2+ female group was as high as the mean tPA level detected in males. However, due to the small size of the ϵ2+ female population (n = 4), further studies will be needed to consolidate this observation.

We demonstrated that there exists no correlation between ApoE genotype and circulating tPA and PAI-1 levels in healthy, resting individuals. It is noteworthy that this study was limited to a middle-aged population. Since tPA and PAI-1 plasma levels increase with age and since age is a major risk factor for AD, it may be important to examine the influence of the apoE genotype on the plasma level of both proteins in older donors.

We cannot rule out that an impact of apoE4 on tPA/PAI-levels may be restricted to the CNS. An answer to this may be given by a study similar to that described here, but using cerebrospinal fluid from healthy patients. Also, in AD brains, the specific interaction between apoE4 and β amyloid [Strittmatter et al., 1993] could lead to the formation of complexes blocking LRP function, a phenomenon particularly deleterious in cognitive regions of the brain where tPA may regulate plasticity.

In summary, there is no significant impact of apoE4 on blood tPA-, PAI-1-antigen, and α2 macroglobulin plasma levels in middle-aged, healthy individuals at rest, and we therefore conclude that the well-documented risk of the apoE4 allele for AD and CHD does not involve alterations in circulating levels of tPA, PAI-1, or α2 macroglobulin.

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TABLE III. Effect of ApoE Genotypes on Protein Levels Stratified by Gender†

Males	All	ϵ2+	ϵ2–	ϵ4+	ϵ4–
tPA (ng/ml)	8.6 ± 3.2	9.2 ± 3.6	8.6 ± 3.2	8.7 ± 2.3	8.7 ± 3.4
PAI-1 (ng/ml)	13.9 ± 12.3	13.8 ± 10.8	13.4 ± 12.8	11.0 ± 4.7	14.8 ± 13.1
α2M (μM)	4.2 ± 0.9	4.5 ± 1.1	4.1 ± 0.8	4.1 ± 0.7	4.2 ± 0.9
Females	**All**	**ϵ2+**	**ϵ2–**	**ϵ4+**	**ϵ4–**
tPA (ng/ml)	5.6 ± 2.6	10.3 ± 3.6*	5.3 ± 2.2	5.7 ± 2.6	5.6 ± 2.6
PAI-1 (ng/ml)	7.2 ± 6.9	23.8 ± 16.2**	6.1 ± 4.3	9.2 ± 9.8	6.4 ± 5.2
α2M (μM)	4.9 ± 0.9	4.2 ± 0.6	4.9 ± 0.9	4.8 ± 1.0	4.9 ± 0.9

†Values are mean ± SD. tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; α2M, alpha 2 macroglobulin.
*n = 4, $P = 0.0181$.
**$P = 0.0249$.

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